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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/058,323	04/09/98	HOUWEN B	10690/101683

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EXAMINER	
GABEL, G	

ART UNIT	PAPER NUMBER
1641	2

DATE MAILED: 09/28/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/058,323	Houwen et al.
Examiner	**Group Art Unit**
Gailene R. Gabel	1641

☒ Responsive to communication(s) filed on *Jun 11, 1998* .

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-12* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-12* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *2*

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Priority

1. It is noted that this application appears to claim subject matter disclosed in prior copending Application No. 09/019,932, filed February 6, 1998. A reference to the prior application must be inserted as the first sentence of the specification of this application if applicant intends to rely on the filing date of the prior application under 35 U.S.C. 119(e) or 120. See 37 CFR 1.78(a). Also, the current status of all nonprovisional parent applications referenced should be included.

Oath/Declaration

2. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because all inventors failed to indicate the date of signing of the declaration.

Drawings

3. The drawings in this application are objected to by the Draftsperson (see PTO-948 attached) Correction is required. However, formal correction of noted defect can be deferred until application is allowed by the examiner.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 2-12 have improper antecedent basis problems by reciting "A method according to claim...". Change to --The method according to claim ...--, for proper antecedent basis Claim 1 (I) is indefinite in reciting "capable of binding specifically with leucocytes" because it fails to recite a positive limitation in the claim. See also claim 11.

Claim 1 (i) is indefinite and confusing in reciting "capable of binding specifically with leucocytes to the hematologic sample" because it fails to recite a positive limitation in the claim. Furthermore, it suggests that the labeled antibody binds the leucocytes to the hematologic sample which does not appear to be applicants intent. It appears that "to the hematologic sample" should really be --in the hematologic sample--.

The term "usually" in claim 1 (ii), line 8 is a relative term which renders the claim indefinite. The term "usually" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Furthermore, claim 1 is confusing in reciting

"which does not permeate a cell membrane usually" because it is unclear as to whether applicants intend to mean that the nucleotide fluorescent dye selectively permeates certain hematologic cell membranes (usually) or that the nucleotide fluorescent dye selectively permeates erythroblast cell membranes only.

Claim 1 (ii), line 9 is indefinite in reciting "capable of being distinguished from that of a fluorescent labeling compound" because it fails to recite a positive limitation in the claim. See also claims 2 and 7.

In claim 1 (iv), line 14, change "flowcytometry" to --flow cytometry--. See also claim 10.

Claim 1 (iv) is indefinite in reciting "subjecting the hematologic sample to flow cytometry to detect at least two fluorescent signals from each cell" because the term "subjecting" does not specifically define what procedural step the claim intends to encompass, i.e. analyzing the hematologic sample. See also claim 10. Furthermore, it does not specify which particular cells in the hematologic sample are encompassed by "each cell", i.e. erythrocytes, erythroblasts, reticulocytes, leucocytes, thrombocytes, myeloblasts, etc.

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential structural cooperative relationships of elements, such omission amounting to a gap between the necessary structural connections. See MPEP § 2172.01. The claim fails to specifically define how the varying intensities compare between erythroblasts and "other cell group" so as to allow discrimination and counting of these cells.

Claim 2 has improper antecedent basis in reciting "leucocytes", first and second occurrence. Change to --the leucocytes-- first and second occurrence, for proper antecedent basis. See also claim 4 (2) and 5.

Claim 3 is indefinite by reciting "CY5". Acronyms or abbreviations must be recited at least one time in a set of claims.

Claim 3 lacks antecedent support by reciting "the fluorescent labeling compound".

In claim 4, change numericals from "(1)" and "(2)" to --(I)-- and --(ii)-- for consistency. (See claim 1).

Claim 4 is non-idiomatic and , therefore, confusing in reciting "only" because it is unclear as to whether the claim intends to "raise the *permeability only* of cell membranes" or "permeability only of cell membranes *of erythroblasts*".

Claim 4 has improper antecedent basis problem in reciting "cell membranes of erythroblasts". Change to --the cell membranes of erythroblasts-- for proper antecedent basis.

The term "suitable", first and second occurrence in claim 4 (2) is a relative term which renders the claim indefinite. The term "suitable" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Furthermore, claim 4 (2) is indefinite in reciting "a pH suitable for staining" because it does specify which cellular element and portion thereof is being stained, i.e. erythroblast nuclei.

Claim 7 is indefinite in reciting "the at least two fluorescent signals detected for each cell" because it does not specifically define which particular cells in the hematologic sample are encompassed by "each cell" which produce the "two fluorescent signals", i.e. erythrocytes, erythroblasts, reticulocytes, leucocytes, thrombocytes, myeloblasts, etc.

Claim 8 is vague and has improper antecedent basis in reciting *"an area* in which erythroblasts appear". Change to --an area in which the erythroblasts appear--, for proper antecedent basis. Furthermore, as recited, it is unclear as to what other cells, likewise, appear in the area where erythroblasts appear and as to whether *other "specific" areas* on the two-dimensional chart is specific to erythroblasts (is there more than one erythroblast area). See also claims 9, 11 and 12. Please clarify.

Claim 9 has improper antecedent basis problem in reciting "leukocytes and erythroblasts". Change to --the leucocytes and the erythroblasts--, for proper antecedent basis. See also claim 11.

Claim 10 is confusing and has improper antecedent basis problem in reciting "in a mixture". Change to --to form the mixture--, for proper antecedent basis and clarity.

In claim 11, change numericals from "(1)" and "(2)" to --(I)-- and --(ii)-- for consistency. (See claim 1).

In claim 12, line 5, change "an total erythroblast count" to -- a total erythroblast count-- for proper grammar.

In claim 12, line 9, add --are obtained.-- after "all the erythroblasts" to complete the claim.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 1-3 and 5- 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,559,037) in view of Loken et al. (US 5,047,321).

Kim et al. disclose a method for the simultaneous and quantitative, flow cytometric analysis of erythroblasts (nucleated red blood cells or nRBC) and leucocytes (white blood cells or WBC). Kim et al. specifically disclose raising cytoplasmic permeability of nucleotide

fluorescent dye (vital nuclear stain) to erythroblasts while minimizing its permeation into leucocytes (see column 2, lines 40-48). Kim et al. teach mixing an aliquot of the blood sample with diluent which rapidly destroys the cytoplasm (lyses) erythroblasts and erythrocytes allowing exposure of erythroblastic nuclei while preserving the integrity and shape of the cytoplasm of leucocytes (see column 4, lines 60-65). The nucleotide fluorescent dyes used by Kim et al. include YOYO-1, YOYO-3, TOTO-1, TOTO-3, BO-PRO-1, YO-PRO-1, TO-PRO-1, Propidium iodide, ethidium bromide, etc depending on the appropriate light source used (see column 6, lines 36-57). Kim et al. constructs a three-dimensional plot of qualified intensity signals of fluorescence and scattered light from detected signals to differentiate and quantitate erythroblasts and leucocytes after flow cytometric analysis. Kim et al. fail to disclose staining of leucocytes using fluorescent-labeled antibody which specifically binds leucocytes in a hematologic sample.

Loken et al. disclose a multiparameter analysis of cells in a body fluid, i.e. whole blood sample, comprising at least two nucleotide fluorescent dyes (nucleic acid dyes) and at least one fluorescent labeled antibody (fluorescently labeled cell surface marker) specific for leucocytes (nucleated hematopoietic cells) (see column 4, lines 21-40). Loken et al. specifically teach that the dyes will independently and differentially assess different characteristics of cells in the sample and the fluorescent labeled antibody recognizes an antigen that is differentially expressed in cells of different lineages (see column 2, lines 37-67). Each of the dye and label is fluorescent, excitable at the same wavelength and has a peak emission spectra that is distinguishable from the others. The fluorescent labeled antibody, i.e. CD45 Mab such as HLe-1, is a monoclonal

antibody that will selectively attach to a cell surface antigen i.e. CD45 antigen, that is expressed in hematopoietic cells in different amounts, various types, and maturational stages of nucleated cells. Loken et al. specifically use phycoerythrin (PE) fluorescent label (see column 5, lines 16-20). The labeled hematologic mixture is measured and analyzed using flow cytometric measurements of fluorescence intensity and light scatter for each cell examined.

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of Loken et al. in differentiating nucleated hematopoietic cells and subclasses using nucleic acid dyes and fluorescent labeled antigen specific antibodies with the teachings of Kim et al. in differentially identifying erythroblasts using nucleotide fluorescent dyes because it allows for simultaneous differentiation and counting of both erythroblasts and leucocytes. One of ordinary skill in the art would have been motivated to incorporate the teachings of Loken et al. in multiparameter analysis and identification of different hematologic nucleated cells into the different nucleotide fluorescent dyes and permeability techniques as taught by Kim et al. because it allows for reduced analysis time coupled with greater accuracy in discriminating different cellular types.

6. Claims 1-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Inami et al. (US 5,298,426) in view of Loken et al. (US 5,047,321).

Inami et al. disclose a two-step method of differentiating erythroblasts from leucocytes. Inami et al. specifically disclose mixing blood with a hypotonic fluorescent dye solution capable

of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic range. Inami et al. further mixes the (acidic) sample mixture with a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained (see column 2, lines 3-24 and column 4, lines 17-41). The first acidic and hypotonic fluid has a low osmolality causing erythrocytic cell lines in the sample to swell upon absorbing water causing cellular contents to leak out and nucleotide fluorescent dye (erythroblastic dye to diffuse through the cell membrane to stain their nuclei. Leucocytes do not permit the entrance of nucleotide fluorescent dyé (see column 5, line 60 bridging to column 6, line 26). Inami et al. enumerates the different dyes used in the first fluid for differentiating leucocytes and erythroblasts, including propidium iodide and ethidium bromide specific for erythroblast nuclei, and appropriate concentrations thereof in column 3 of the disclosure. Inami et al. disclose that a the concentration of nucleotide fluorescent dye, i.e. propidium iodide or ethidium bromide, should fall within the range of 0.003 mg/L to 10 mg/L (2.5 μg/ml to 100μg/ ml) in order to achieve optimum results (see column 4, lines 5-16). After treatment, stained cells are measured using a flow cytometer and erythroblasts are separated from other cell groups on the resulting two-dimensional plot where erythroblasts are counted (see column 6, lines 9-12). Figure 9 shows a two-dimensional plot showing selective staining of erythroblasts with nucleotide staining dye to emit red fluorescence and to permit erythroblasts to be distributed in a separate zone from other cells so that the relative content and count can be

determined . Figure 10 and 11 show two-dimensional plots for the intensity of red fluorescence versus the intensity of side-scattered light obtained for peripheral blood and bone marrow. Inami et al. fail to disclose staining of leucocytes using fluorescent-labeled antibody which specifically binds leucocytes in a hematologic sample.

Loken et al. has been discussed supra.

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of Loken et al. in differentiating nucleated hematopoietic cells and subclasses using nucleic acid dyes and fluorescent labeled antigen specific antibodies with the two-step staining method as taught by Inami et al. in treating hematologic blood samples for differentially identifying erythroblasts using nucleotide fluorescent dyes because it allows for simultaneous differentiation and counting of both erythroblasts and leucocytes. One of ordinary skill in the art would have been motivated to incorporate the teachings of Loken et al. in multiparameter analysis and identification of different hematologic nucleated cells into the specialized staining technique in staining erythroblast nuclei as taught by Inami et al. because it allows for reduced analysis time coupled with greater accuracy in discrimination and counting of erythroblasts in peripheral blood which are specific diagnostic indicators of diseases such as anemia and leukemia.

7. For reasons aforementioned, no claims are allowed.

Remarks

8. Prior art made of record are not relied upon but considered pertinent to the applicants' disclosure:

Sakata et al. (US 5,496,734) disclose rapid treatment of a blood sample so that it can be analyzed for counting and classification of leucocytes.

Bianch (US 5,641,628) disclose isolation and detection of fetal DNA from maternal blood (see column 6, line 38 to column 8, line 61).

Leif et al. (US 5,188,935) disclose a reagent system and method foe identification and enumeration/ examination of classes and subclasses of blood leucocytes.

Chopp et al. (US 5,939,326) disclose a multipurpose reagent system suitable for rapid analysis of nucleated peripheral blood cells including leucocytes and erythroblasts (see column 12 to column 16).

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gail Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday from 7:00 AM to 4:30 PM. The examiner can also be reached on alternate Fridays from 7:00 AM to 3:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James Housel, can be reached on (703) 308-4027. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

9/23/99

Gail Gabel
Patent Examiner
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